

A Novel Filtration-Based Processing Method of Liquid Cytology Specimens for Human Papillomavirus DNA Testing by Hybrid Capture II

Philip E. Castle, PhD, MPH,¹ Mariane Garcia-Meijide, MS,^{2*} E. Blair Holladay, PhD,³ Ronald Chuke, MS,^{2*} Jay Payne, PhD,^{2*} Amanda Long,⁴ Heather Siefers,⁴ Franklin Demuth,⁵ and Attila T. Lorincz, PhD^{2*}

Key Words: Human papillomavirus; HPV; Hybrid Capture II; PreservCyt

DOI: 10.1309/MEPB1TAMAD6Q13G3

Abstract

We evaluated a more efficient method of processing liquid-based cervical cytology specimens for human papillomavirus (HPV) DNA testing by Hybrid Capture II (HCII). Aliquots were made from 701 specimens in the following sequence: 4.0, 2.0, 1.0, 0.5, and 1.5 mL. The 4.0-mL aliquot was processed by the standard method (STP), and half of the processed material was tested by HCII. Other aliquots were processed with a new, filtration-based processing method (NPM). The 2.0-mL NPM aliquot had HCII test performance most similar to the STP, ie, similar HCII positivity ($P = .4$) and good test agreement ($\kappa = 0.85$, 95% confidence interval [CI], 0.80-0.89). The 194 cytologic negatives had greater positivity by STP ($P = .04$) compared with the 2.0-mL aliquot processed by NPM; between-method agreement was modest ($\kappa = 0.54$, 95% CI, 0.36-0.72). A lower positive cut point for the 2.0-mL NPM aliquot partially abrogated this minor difference. In 241 specimens diagnosed as low-grade and 31 as high-grade squamous intraepithelial lesions, there were no significant differences in HPV positivity (>85% and 90%, respectively) between STP and NPM. NPM reduces specimen handling and decreases total testing time by approximately 33% without significant losses in HCII test performance.

Cervical infections by approximately 15 cancer-associated (oncogenic) human papillomavirus (HPV) types cause virtually all cervical cancer worldwide.¹⁻³ Most oncogenic HPV infections are transient, but occasionally these infections persist. It now is recognized that persistent oncogenic HPV infection precedes and is a critical step in the development of cervical cancer or its immediate precursor (cervical intraepithelial neoplasia [CIN] grade 3).⁴

HPV DNA testing is a more sensitive but less specific alternative to cytologic screening.^{5,6} Moreover, there are ample prospective data to suggest that a single baseline HPV DNA test is more sensitive than the single conventional Papanicolaou smear for the detection of CIN3 or cancer (\geq CIN3) during a 5- to 10-year period.^{7,8} Consequently, HPV DNA negativity suggests a very low risk of prevalent or incipient cancer or CIN3.⁸ One randomized trial demonstrated that HPV DNA testing is a useful triage of equivocal, borderline, or atypical squamous cells (ASC) of unknown cytologic significance.⁹ Accordingly, HPV testing has been approved in the United States as a reflex or follow-up test to cytology for triage of ASC and for adjunctive screening with cytology in women 30 years old or older.¹⁰⁻¹²

Hybrid Capture II (HCII; Digene, Gaithersburg, MD) using probe set B is a pooled probe test for detection of 13 oncogenic HPV DNA types. HCII relies on the formation of target HPV DNA-RNA probe heteroduplexes during the hybridization step in specimens positive for one or more oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) and the detection of these hybrids by using an alkaline phosphatase-conjugated monoclonal antibody specific to DNA-RNA complexes with dioxetane substrate in a 96-well chemiluminescent enzyme-linked immunosorbent assay format. Signal strengths in relative

light units (RLU) are compared with an RLU cutoff (CO) value for the 1-pg/mL HPV type 16 DNA positive control samples (RLU/PC), and 1.0 RLU/CO (~1 pg/mL) or more is considered a positive result.^{13,14}

HCII can conveniently use residual cells in liquid-based cytologic medium after the preparation of a cytologic slide. One example is the use of residual ThinPrep/PreservCyt specimens (Cytec, Boxborough, MA) for HCII testing.¹⁵ The US Food and Drug Administration (FDA)-approved method is to use a 4.0-mL aliquot of residual PreservCyt specimens, process the aliquot resulting in cell lysis and release of viral DNA, and use half of the resulting lysate in the HCII assay (eg, an equivalent of 2 mL or 10% of the original specimen is used for signal detection).

One difficulty for this assay method is the laborious and time-consuming processing of the PreservCyt specimen aliquots. It takes approximately 10.5 hours to process 352 PreservCyt specimens (4 HCII testing 96-well plates) with the standard FDA-approved method (STP). With a new, filtration-based processing method (NPM) developed by Digene, it takes approximately 7 hours to process the same 352 PreservCyt specimens, decreasing the total time for processing by 33% or more. Following specimen processing, the HCII HPV test requires an additional 4.5 hours. However, this new processing method has not been validated against the STP. Thus, we sought to compare the NPM with the STP to determine their concordance. We also evaluated whether a smaller volume might be used in the NPM to reduce specimen requirements and minimizing clogging of the filtration system.

Materials and Methods

Specimens

A convenience sample of 701 PreservCyt specimens was acquired from the Medical University of South Carolina, Charleston, after routine use for cytologic screening. Cytologic interpretations were classified as negative ($n = 194$), ASC ($n = 234$), low-grade squamous intraepithelial lesion (LSIL; $n = 241$), or high-grade squamous intraepithelial lesion or cancer (\geq HSIL; $n = 31$). One specimen, interpreted as atypical glandular cells, was classified as “other.” Specimens were stripped of identifiers to preserve anonymity. The study was approved by the local institutional review board (Medical University of South Carolina) and was deemed exempt from review by the National Institutes of Health (Bethesda, MD). On their arrival at the National Cancer Institute–contracted biorepository (BBi Biotech Research Laboratories, Gaithersburg, MD), PreservCyt specimens were stored at controlled ambient temperature until used.

Specimen Processing

Five aliquots were made from each PreservCyt specimen with vigorous mixing before and between making each aliquot in the following order: one 4.0-mL aliquot for the STP using the Digene PreservCyt Specimen sample conversion protocol and 4 subsequent aliquots of 2.0, 1.0, 0.5, and 1.5 mL for the NPM **Figure 1**. One test group was made up of 18 PreservCyt specimens (90 aliquot tubes). Each patient sample aliquot (with various volumes for testing) received unique identifiers to ensure that all processing and testing was performed at Digene in a masked manner.

All aliquot samples were stored at 4°C or processed on the day of delivery. The order of processing per sample group for the first 701 PreservCyt specimens was as follows: box 1 (18 4.0-mL samples) was processed first using the Digene PreservCyt Specimen sample conversion protocol as described in subsequent text; box 2 (18 2.0-mL samples) and box 3 (18 each 0.5-mL and 1.5-mL samples) were processed together, starting with the 2.0-mL samples, the 0.5-mL samples next, and the 1.5-mL samples last, using the NPM. Four aliquot sample groups were processed (day 1) and assayed (day 2) together per week throughout the study.

The 4.0-mL aliquot of PreservCyt was processed as previously reported.^{13,16} Briefly, a proprietary sample conversion buffer (Digene) was added (400 μ L) to each specimen and vigorously mixed. Aliquots then were centrifuged to pellet cellular content, and supernatant was decanted carefully to avoid disruption of the pellet. After HCII denaturing solution was added (150 μ L) to each pellet of all 4.0-mL samples, the pellet was suspended by vigorous mixing and incubated for 15 minutes at 65°C, then by mixing again, and then placed back into a 65°C water bath for an additional 30 minutes.

For the NPM, each aliquot was mixed for 10 seconds, and the entire contents of each aliquot were transferred to the designated well on a deep-well filtration plate (Figure 1). Once all samples were transferred to the filtration plate, the plate was centrifuged to collect sample cellular content on the filter, and the PreservCyt filtrate was discarded. Next, 75 μ L of Digene denaturing solution was added to each sample well on the filtration plate; the plate was mixed using the Digene plate shaker and then placed into a custom heat block set at 65°C for 45 minutes. After denaturation, the filter plate was allowed to cool and then centrifuged to collect denatured samples in a microtiter plate. (Note: All samples within a batch received the same denaturation time.) Once centrifugation transfer of denatured material to a microtiter plate was completed, 75 μ L of denatured material from each of the 4.0-mL samples was transferred to its designated well on the microtiter plate harboring the filtered-denatured samples. The microtiter plate harboring all denatured material from an aliquot sample group was placed at –20°C overnight for assay the next day.

The Digene Rapid Capture System was used to assay all aliquot samples tested for the entire study following the standard HPV HCII probe B assay protocol.

Statistical Analysis

To compare the NPM for each aliquot volume with the STP, Spearman correlation coefficients were calculated for all measurements and for test positives (≥ 1.0 RLU/CO), κ values with 95% confidence intervals (95% CI) were calculated as a measure of test agreement, and McNemar χ^2 values were calculated to test for statistical differences ($P < .05$) in test positives (sensitivity). Results also were stratified by cytologic interpretation.

Finally, we examined whether using lower positive cut points (0.90, 0.80, and 0.70 RLU/CO) for the 2.0-mL aliquot processed by the NPM might result in better agreement with the STP.

Results

A summary of the test results is shown in Table 1. Despite the lower test positivity, the median test value among paired test positives was significantly greater for the 2.0-mL aliquots processed by the NPM than the STP ($P = .03$; Kruskal-Wallis). The correlations between the STP and the NPM for all aliquots were 0.90 or more for all tests and 0.96 or 0.97 among the paired test positives.

Overall, there was very good agreement in test positivity between the STP and all aliquots with the NPM, with κ values all above 0.80 Table 2. The STP was more likely to be test positive than all NPM aliquots ($P < .05$). Among the cytologically negative samples, the agreement between NPM and STP was the worst, with κ values ranging from 0.41 (1.0-mL aliquot) to 0.63 (1.5-mL aliquot). Among the ASC, LSIL, and HSIL samples, there was good or better agreement ($\kappa \geq 0.73$) and no significant differences in test positivity between the STP and the 2.0-, 1.0-, and 1.5-mL aliquots processed using the NPM. The

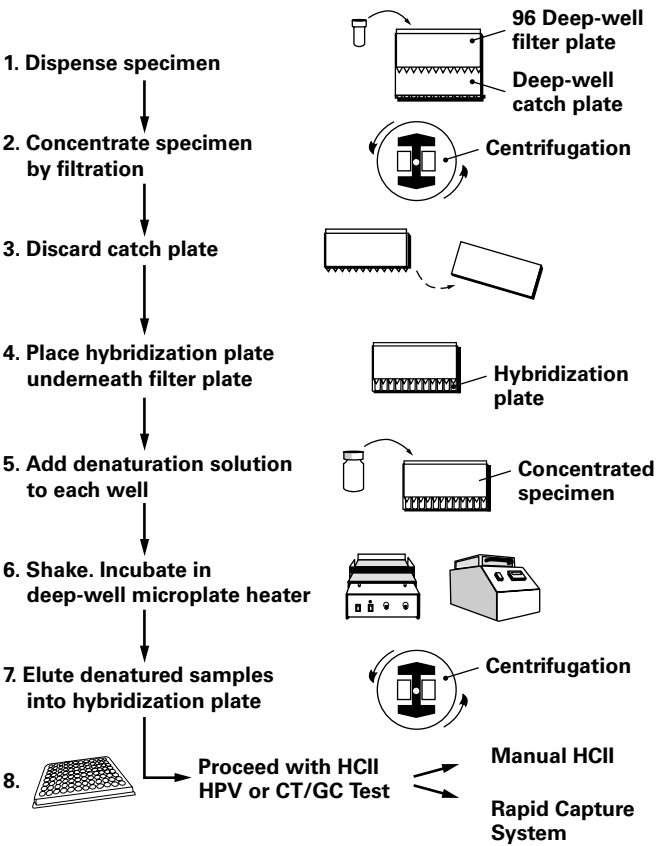


Figure 1 Schematic for the novel processing method (NPM) for PreservCyt liquid cytologic specimens for Hybrid Capture II (HCII) testing for human papillomavirus (HPV) DNA detection and for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection (CT/GC). For proprietary information, see the text.

0.5-mL aliquot was less positive than the STP among ASC ($P = .005$), LSIL ($P = .03$), and HSIL ($P = .3$) samples.

Finally, we examined whether using lower positive cut points for 2.0-mL aliquots for the NPM could be used to better approximate STP test results Figure 2. Lowering the positive cut point for the 2.0-mL NPM to 0.90, 0.80, and 0.70

Table 1 Hybrid Capture II Test Statistics and Spearman Correlations for the STP and NPM

	No. Positive	Positivity (%)	Test Positives (≥ 1 RLU/CO)			Correlations of RLU/CO NPM vs STP	
			Mean	Median	Range	All	Positives*
STP	378	55.6	394.7	101.2	1.0-2,637.8	—	—
NPM volume (mL) [†]							
2.0	371	54.6	556.9	181.0	1.0-3,457.2	0.93	0.96
1.0	360	52.9	464.2	91.8	1.0-3,831.6	0.92	0.96
0.5	345	50.7	367.0	61.8	1.0-3,557.5	0.91	0.96
1.5	358	52.6	498.9	126.1	1.0-3,825.5	0.94	0.97

CO, cutoff; NPM, new filtration-based processing method; RLU, relative light units; STP, standard US Food and Drug Administration–approved method.

* Both tests were above the positive cut point, ie, ≥ 1.0 RLU/CO.

[†] Of PreservCyt. For proprietary information, see the text.

Table 2

Comparison of Test Results for STP and NPM Using Different Volumes of PreservCyt for All Specimens and Results Stratified by Cytologic Category*

	STP/NPM Result							
NPM Volume (mL)	+/NA	NA/+	-/-	+/-	-/+	+/+	κ (95% CI)	P [†]
All								
2.0 (n = 697)	394 (56.5)	385 (55.2)	282 (40.5)	30 (4.3)	21 (3.0)	364 (52.2)	0.85 (0.81-0.89)	.2
1.0 (n = 701)	397 (56.6)	375 (53.5)	285 (40.7)	41 (5.8)	19 (2.7)	356 (50.8)	0.83 (0.79-0.87)	.005
0.5 (n = 701)	397 (56.6)	359 (51.2)	290 (41.4)	52 (7.4)	14 (2.0)	345 (49.2)	0.81 (0.77-0.85)	<.001
1.5 (n = 684)	381 (55.7)	361 (52.8)	290 (42.4)	33 (4.8)	13 (1.9)	348 (50.9)	0.86 (0.83-0.90)	.003
Cytologically negative								
2.0 (n = 194)	28 (14.4)	19 (9.8)	161 (83.0)	14 (7.2)	5 (2.6)	14 (7.2)	0.54 (0.36-0.72)	.04
1.0 (n = 194)	28 (14.4)	15 (7.7)	161 (83.0)	18 (9.3)	5 (2.6)	10 (5.2)	0.41 (0.21-0.60)	.007
0.5 (n = 194)	28 (14.4)	15 (7.7)	163 (84.0)	16 (8.2)	3 (1.5)	12 (6.2)	0.51 (0.32-0.70)	.003
1.5 (n = 190)	25 (13.2)	14 (7.4)	164 (86.3)	12 (6.3)	1 (0.5)	13 (6.8)	0.63 (0.45-0.81)	.002
ASC								
2.0 (n = 231)	130 (56.3)	126 (54.5)	93 (40.3)	12 (5.2)	8 (3.5)	118 (51.1)	0.82 (0.75-0.90)	.4
1.0 (n = 234)	132 (56.4)	125 (53.4)	93 (39.7)	16 (6.8)	9 (3.8)	116 (49.6)	0.78 (0.70-0.86)	.2
0.5 (n = 234)	132 (56.4)	117 (50.0)	95 (40.6)	22 (9.4)	7 (3.0)	110 (47.0)	0.75 (0.67-0.84)	.005
1.5 (n = 228)	126 (55.3)	119 (52.2)	96 (42.1)	13 (5.7)	6 (2.6)	113 (49.6)	0.83 (0.76-0.90)	.1
LSIL								
2.0 (n = 240)	208 (86.7)	212 (88.3)	24 (10.0)	4 (1.7)	8 (3.3)	204 (85.0)	0.77 (0.65-0.90)	.2
1.0 (n = 241)	209 (86.7)	207 (85.9)	27 (11.2)	7 (2.9)	5 (2.1)	202 (83.8)	0.79 (0.67-0.90)	.6
0.5 (n = 241)	209 (86.7)	200 (83.0)	28 (11.6)	13 (5.4)	4 (1.7)	196 (81.3)	0.73 (0.60-0.85)	.03
1.5 (n = 235)	203 (86.4)	201 (85.5)	26 (11.1)	8 (3.4)	6 (2.6)	195 (83.0)	0.75 (0.63-0.88)	.6
HSIL or more								
2.0 (n = 31)	28 (90)	28 (90)	3 (10)	0 (0)	0 (0)	28 (90)	1.0	1.0
1.0 (n = 31)	28 (90)	28 (90)	3 (10)	0 (0)	0 (0)	28 (90)	1.0	1.0
0.5 (n = 31)	28 (90)	27 (87)	3 (10)	1 (3)	0 (0)	27 (87)	0.84 (0.53-1.0)	.3
1.5 (n = 30)	27 (90)	27 (90)	3 (10)	0 (0)	0 (0)	27 (90)	1.0	1.0

ASC, atypical squamous cells; CI, confidence interval; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NA, not applicable;

NPM, new filtration-based processing method; STP, standard US Food and Drug Administration-approved method; +, positive; -, negative.

* Data are given as number (percentage). For proprietary information, see the text.

† McNemar χ^2 test vs STP.

RLU/PC increased the overall positivity to 56.5% ($P = 1.0$ vs STP), 58.1% ($P = .1$ vs STP), and 59.8% ($P = .003$ vs STP), respectively, compared with 56.5% positivity for the STP and 55.2% positivity for the 2.0-mL NPM at a 1.0 RLU/PC positive cut point. The κ values for the different cut points were between 0.82 and 0.85 and were not statistically distinguishable. Lowering the positive cut point for the 2.0-mL NPM did not affect the positivity for HSIL or more. Using a 0.90 RLU/PC cut point resulted in lower test positivity among cytologically negative samples (10.8% vs 14.4%; $P = .1$) and similar test positivity for ASC (56.3% vs 57.1%) and LSIL (86.7% vs 88.8%) compared with the STP. Using a 0.80 RLU/PC cut point resulted in similar test positivity among cytologically negative samples (11.9% vs 14.4%) and elevated positivity in ASC (56.3% vs 59.7%; $P = .1$) and LSIL (86.7% vs 90.0%; $P = .03$) compared with the STP.

Discussion

We compared HCII test results using 2 PreservCyt specimen processing techniques, the FDA-approved method (STP) and the prototype novel, filtration-based processing method

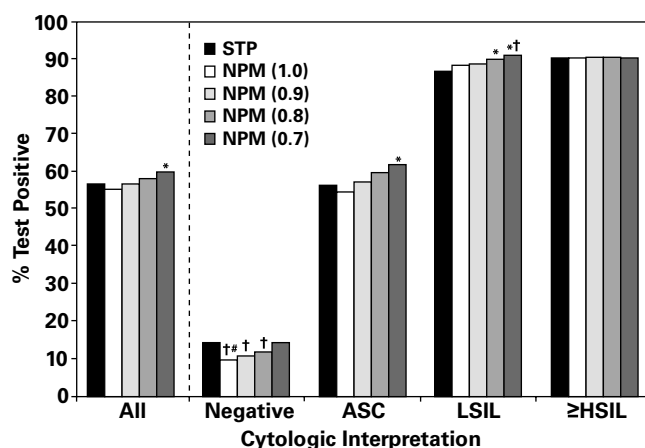


Figure 2 A comparison of Hybrid Capture II (HCII) test positivity for the standard test procedure (STP) with a 1.0 relative light units (RLU)/cutoff (CO) positive cut point and HCII test positivity for 2.0-mL aliquots processed using the new, filtration-based method (NPM) with different positive cut points (1.0, 0.90, 0.80, and 0.70 RLU/CO). All results combined and results stratified on cytologic category are shown. * Increased test positivity compared with the STP ($P < .05$; McNemar χ^2). † values for HCII test positivity <0.70 compared with the STP. ‡ Decreased test positivity compared with the STP ($P < .05$; McNemar χ^2). For proprietary information, see the text.

(NPM) designed to automate and reduce specimen-processing time. Overall, we found very similar HCII test performance and very good concordance between the STP, which uses a 2.0-mL aliquot equivalent, and a 2.0-mL aliquot processed by the NPM. However, the HCII test performance using smaller volumes gave a lower percentage of HPV positive tests and did not seem equivalent to the STP.

We observed reduced HCII positivity for 2.0-mL NPM compared with the STP, primarily among women with negative cytologic results. We suggest that there is a systematic loss of positive test results using the NPM, which would be expected to preferentially affect HCII test results among cytologically normal women who will have the lowest signals (ie, viral loads). Conversely, equivocal and mild cytologic changes are synonymous with productive viral infections, higher viral loads, and, therefore, HCII testing of these specimens has a greater signal strength⁵ that will be less negatively affected by small losses in total viral genomes. We noted that the signals of more than 1.0 RLU/PC for paired positive tests were greater for the 2.0-mL NPM than for the STP, suggesting that there might be some compensatory increased efficiency in viral genome recovery and that there is a different dynamic curve for HCII testing for the NPM than for the STP.

We emphasize that reduced HCII test positivity was among cytologically negative women who are very unlikely (<1%) to have cervical cancer or precancer.⁸ Using a slightly lower threshold for positivity partially offset this loss in test positivity. However, it is possible that using the 2.0-mL NPM at the 1.0 RLU/PC cut point resulted in a more clinically specific assay without lowering the sensitivity. One important limitation in our study is that we did not have data for comparing HCII test results with histologic diagnoses of CIN3 and cancer. Nor did we have follow-up data to overcome insensitivity of colposcopic evaluations and directed biopsies for complete disease ascertainment.¹⁷ Future evaluations will need to incorporate complete disease ascertainment to determine the true performance using NPM.

A second limitation is that the PreservCyt specimens were older than the 30 days recommended for clinical HCII testing. Thus, it is conceivable that signal degradation occurred because of specimen aging. However, we used paired aliquots in masked testing, thus internally controlling for aging of specimens. There is no a priori reason to suspect that specimen aging might preferentially affect one processing method compared with the other method, although we cannot completely discount this possibility. We also noted that the HCII positivity rate for each cytologic interpretation was consistent with other reports, eg, ASC and LSIL were approximately 50% and 80% HCII-positive, respectively, as previously reported.^{9,16}

It is noteworthy that the HCII positivity for the 1.5-mL NPM was similar to the 1.0-mL NPM, 52.6% vs 52.9%, rather

than having HCII positivity that was intermediate between 1.0-mL and 2.0-mL (54.6%) aliquots. For negative and ASC cytologic results, test positivity was lower for the 1.5-mL than for the 1.0-mL aliquots. These data suggest that there might be order effect for testing performance and might partially explain the aforementioned systematic error.

HCII testing using NPM processing of a 2.0-mL aliquot of residual PreservCyt specimens had similar test performance to HCII testing using the STP method. There was a loss in HCII positivity among cytologically negative samples for the 2.0-mL NPM that can be partially compensated for by using a slightly lower positive cut point. It is unclear whether this lower positivity in cytologically negative samples has clinical consequences for detection of CIN3 or cancer, ie, decreased sensitivity or increased specificity, in a group of women who rarely will have those histologic diagnoses. Future evaluations are needed incorporating full disease assessment to address this issue. It is important to note that this processing method increases the testing efficiency of PreservCyt aliquots, which will become an important consideration as adjunct HPV testing becomes routine in cervical cancer screening.

From the ¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; ²Digene, Gaithersburg, MD; ³Medical University of South Carolina, Charleston; ⁴BBI Biotech Research Laboratories, Gaithersburg, MD; and ⁵Information Management Services, Silver Spring, MD.

Address reprint requests to Dr Castle: Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, MSC 7234, Bethesda, MD 20892-7234.

**Attila Lorincz (Chief Scientific Officer), Jay Payne, Ronald Chuke, and Marianne Garcia-Mejide work for Digene, producers of Hybrid Capture II and the new processing method described in this study, and are Digene stockholders.*

References

1. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003;348:518-527.
2. Bosch FX, Manos MM, Munoz N, et al, for the International Biological Study on Cervical Cancer (IBSCC) Study Group. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *J Natl Cancer Inst*. 1995;87:796-802.
3. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 1999;189:12-19.
4. Wright TC Jr, Schiffman M. Adding a test for human papillomavirus DNA to cervical-cancer screening. *N Engl J Med*. 2003;348:489-490.
5. Schiffman M, Herrero R, Hildesheim A, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. *JAMA*. 2000;283:87-93.

6. Cuzick J, Szarewski A, Cubie H, et al. Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet*. 2003;362:1871-1876.
7. Ferreccio C, Bratti MC, Sherman ME, et al. A comparison of single and combined visual, cytologic, and virologic tests as screening strategies in a region at high risk of cervical cancer. *Cancer Epidemiol Biomarkers Prev*. 2003;12:815-823.
8. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. *J Natl Cancer Inst*. 2003;95:46-52.
9. Solomon D, Schiffman M, Tarone R. Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *J Natl Cancer Inst*. 2001;93:293-299.
10. Wright TC Jr, Schiffman M, Solomon D, et al. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obstet Gynecol*. 2004;103:304-309.
11. Saslow D, Runowicz CD, Solomon D, et al. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. *CA Cancer J Clin*. 2002;52:342-362.
12. ACOG Committee on Practice Bulletins, ACOG Practice Bulletin: Clinical Management Guidelines for Obstetrician-Gynecologists, Number 45, August 2003: cervical cytology screening (replaces committee opinion 152, March 1995). *Obstet Gynecol*. 2003;102:417-427.
13. Schiffman M, Adriaan ME. ASCUS-LSIL Triage Study: design, methods and characteristics of trial participants. *Acta Cytol*. 2000;44:726-742.
14. ASCUS-LSIL Triage Study (ALTS) Group. A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. *Am J Obstet Gynecol*. 2003;188:1393-1400.
15. Sherman ME, Schiffman MH, Lorincz AT, et al. Cervical specimens collected in liquid buffer are suitable for both cytologic screening and ancillary human papillomavirus testing. *Cancer*. 1997;81:89-97.
16. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. *J Natl Cancer Inst*. 2000;92:397-402.
17. Guido R, Schiffman M, Solomon D, et al. Postcolposcopy management strategies for women referred with low-grade squamous intraepithelial lesions or human papillomavirus DNA-positive atypical squamous cells of undetermined significance: a two-year prospective study. *Am J Obstet Gynecol*. 2003;188:1401-1405.